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ORIGINAL ARTICLE

p53 codon 72 polymorphism in Taiwanese breast cancer patients

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Abstract There are clear discrepancies between ethnicity and geographic area regarding the peak age incidence and mortality of breast cancer. Underlying variances include genetic, environmental, and socioeconomic factors. The wild-type p53 codon has two common polymorphic variants from a single-base-pair substitution at codon 72, where either C-C-C encodes proline (p53-p72) or C-G-C encodes arginine (p53-R72). We aim to study the p53 codon 72 genotypes of patients with breast cancer in Taiwan and make a comparison with the published data to ascertain whether any difference exists between Taiwanese and Western patients with breast cancer. We also evaluated the effect of the p53 codon 72 polymorphism on clinicopathologic features. We examined blood from 170 Taiwanese women with breast cancer with polymerase chain reaction–restriction fragment length polymorphism for the genotypes of p53 codon 72. For the p53 codon 72 polymorphism, there were 31 p53-P/P72 (18.2%), 93 p53-R/P72 (54.7%), and 46 p53-R/R72 (27.1%) with the allele frequencies 0.54 for the p53-R72 and 0.46 for p53-P72, respectively. Our results indicate that there was more p53-P72 (40.6% in Asians vs. 26.4% in Caucasians) and twice the incidence of p53-P/P72 homozygotes (18% in Asians vs. 8% in Caucasians) among the Asian population. Patients with the p53-R/R72 variant were more

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likely to have a t1 tumor size status (55.2%) compared with patients with the P53-P/R72 (30.9%) or P53-P/P72 variant (36%). Our results support the hypothesis that genetic factors may contribute to the difference between Taiwanese or Asian breast cancer and Western breast cancer patient populations.

Introduction

Among Taiwanese women, breast cancer is the most common form of malignancy and the fourth leading cause of cancer-related death. In 2008, death rates in females from breast cancer were 11.33 per 100,000 people [1]. The risk factors of getting breast cancer in Taiwan, which is a low-incidence area, are similar to those in high- to moderate-risk areas. Family history and the effects of reproductive hormones play the most significant role [2,3]. However, when compared with the number of breast cancer cases in Western countries, the peak of the age-specific incidence rates appeared 10—20 years earlier and the age-specific mortality rates peaked at ages 55—59 years and after age 80 years in Taiwan [1,4]. These divergences may be due to multiple factors including racial/ethnic background and genetic variation [5].

p53, the most frequent genetic event among the tumor suppressors [6,7], exerts a protective response either by regulating the cell cycle at the G1/S checkpoint for DNA repair [8] or by inducing apoptosis in genetically damaged cells [9]. Recently a single nucleotide polymorphism (SNP) of either C-C-C encoding proline (p53-P72) or C-G-C encoding arginine (p53-R72) [10] at codon 72 of the *p53* gene has been extensively studied, not only because of its significant implication in carcinogenesis but also for its clinical controversies. The SNP is located in a proline-rich region (residues 64—92) of the p53 protein, where the p53-P72 amino acid constitutes one of five PXXP motifs that resembles a SH3-binding domain and is required for the growth suppression and apoptosis mediated by p53 [11]. In 1998, Storey et al. [12] showed the unprecedented finding that the E6 proteins from human papilloma virus (HPV) types are able to target p53-R72 more efficiently than p53-P72 for ubiquitin-mediated degradation. They also demonstrated that p53-R/R72 homozygotes are approximately seven times more susceptible to HPV-associated tumorigenesis than heterozygotes. The arginine-encoding allele therefore represents a significant risk factor in the development of HPV-associated cancers [12]. Although subsequent studies further supported the biologic contribution of the codon 72 polymorphism in tumorigenesis [13—17], the significance of the p53 codon 72 polymorphism remains controversial with regard to cancer susceptibility because of the inconsistency of the association between the polymorphism and the cancer risk [16,18—20] or prognosis [20,21] in different cancer types. In breast cancer, p53-R72 has been suggested as a susceptibility allele, especially in Europeans [17,22]. However, p53-P/P72 homozygosity has also been associated with increased breast cancer risk [17,23]. Studies against the association of the p53-R72P polymorphism and breast cancer risk have also been reported [24]. Furthermore, either P53-P/P72 homozygous or retention of the arginine allele in the tumor tissue of p53-R/P72 in patients with heterozygous breast cancer was recently identified as an independent prognostic marker [25,26]. Recent work has also provided evidence that p53 codon 72 polymorphism may modulate the response to cancer therapy [27—29].

The distribution of p53 codon 72 polymorphism as well as the association of this polymorphism with breast cancer features remains undefined in Taiwan, which has a non-Caucasian population with low breast cancer risk. Here we genotyped the p53 codon 72 polymorphism using restriction fragment length polymorphism and compared our genotypes with published results to test if the difference of genotypes exists between different areas.

Methods

Specimen collections

In the current study, blood samples obtained from 170 patients with primary breast cancer who underwent surgery at the Department of Surgery of Kaohsiung Medical University Hospital (KMUH) between February 2003 and February 2007 were analyzed for p53 codon 72 genotyping. This study protocol was approved by the Institutional Review Board of KMUH and informed consent was obtained from each participant. None of the patients had undergone radiotherapy or chemotherapy before surgery. The tumor tissues were sent for routine histopathologic diagnosis, including an HER2 expression study according to the usual criteria at the KMUH, as described previously [30]. Blood samples from control patients without cancer (in a 1:2 ratio) were collected for comparison of the distribution of p53 codon 72 polymorphism with cancer patients.

DNA extraction and p53 codon 72 genotyping

DNA was extracted from the buffy coat using a commercial kit (QIAGEN, Hilden, Germany). Genotyping was performed using the polymerase chain reaction (PCR)—restriction fragment length polymorphism method, with the digested PCR products applied to electrophoresis on agarose gels, and viewed using ethidium bromide. The following primers were used: forward primer 5′-TAATACGACTCACTATAGG GCCGTCCCAAGCAATGGATGAT-3′ and reverse primer 5′-CT GGGAAGGGACAGAAGATGA-3′. PCR was obtained in a 15-μL reaction mixture containing 100 ng of genomic DNA template, 2 μL 10×PCR buffer, 0.8 mmol/L deoxynucleotide triphosphate, 2.5 mmol/L $MgCl_2$, 0.5 μmol/L primers, and 1 unit mpliTaq DNA polymerase (Promega, Madison, MI,

USA). The reaction conditions used were initial denaturation at 94°C for 2 minutes, followed by 35 step-cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 30 seconds followed by a terminal extension time of 10 minutes. Ten microliters of PCR product were digested with BstU1 restriction enzyme (New England Biolabs, Inc., Ipswich, MA, USA) for 12 hours at 60 °C. The digestion products were then resolved on a 2.5% agarose gel containing ethidium bromide. As shown in Fig. 1, the p53-P/P72 variant was identified by a single band (195 bp), the p53-R/R72 variant produced two bands (85 bp and 110 bp), and the heterozygous p53-R/P72 variant displayed three bands (195, 85, and 115 bp).

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences Version 11.0 software (SPSS Inc., Chicago, IL, USA). The two-sided Pearson χ^2 test was used to compare the clinicopathologic parameters with p53 codon 72 genotypes. A probability of less than 0.05 was considered to be statistically significant.

Results

In our study, 170 patients with breast cancer without selection of age or family history were enrolled. The mean age (±standard deviation) for patients with breast cancer was 52.3 (±17.7). There were 20 intraductal carcinomas, 122 infiltrating ductal carcinomas, 17 infiltrating lobular carcinomas, three medullary carcinomas, three tubular carcinomas, three mucinous carcinomas, and two atypical medullary carcinomas. The clinicopathologic characteristics and the frequencies of the three p53 genotypes p53-R/R72, p53-R/P72, and p53-P/P72 are summarized in Table 1. Due to the inadequacy of tumor tissue or missed data, the complete clinicopathologic data were not obtained for every patient. The frequencies of p53-R/R72, p53-R/P72, and p53-P/P72 found in the patients with breast cancer in Taiwan were 27.1% (46 of 170), 54.7% (93 of 170), and 18.2% (31 of 170), respectively. The clinicopathologic features including tumor size, nodal involvement, grading, estrogen receptor (ER), progesterone receptor (PR), and HER2 expression were evaluated. No significant differences in the distribution of clinicopathologic parameters between p53-R72 and p53-P72 patients were found except for tumor size. Patients with the P53-R/R72 variant were more likely to have a t1 tumor size status, compared with patients with the P53-P/R72 or P53-P/P72 variant. In the P53-R/R72 group, 55.2% of patients (16 of 29) were of t1 status compared with 30.9% or 36% of patients (21 of 68 and 9 of 25, respectively) in the P53-P/R72 or P53-P/P72 groups, respectively ($p = 0.046$). Genomic DNA from patients with breast cancer and control patients without cancer (in the 1:2 ratio) was analyzed to compare the distribution of p53 codon 72 polymorphism. Overall, there was no difference in genotype distributions between control patients without cancer and patients with breast cancer (data not shown).

Table 1 Patients' characteristics and clinicopathologic features and p53 codon 72 polymorphism.

	Total, *n*	RR, *n* (%)	RP, *n* (%)	PP, *n* (%)	*p*
Menopausal status					
Premenopausal	59	14 (30.8)	31 (34.2)	14 (41.4)	0.655
Postmenopausal	111	32 (69.2)	62 (65.8)	17 (58.6)	
Tumor histology					
Ductal	129	34 (94.4)	69 (95.8)	26 (86.7)	0.406
Lobular	9	2 (5.6)	3 (4.2)	4 (13.3)	
Tumor size					
t1	46	16 (55.2)	21 (30.9)	9 (36)	0.046
t2+t3+t4	76	13 (44.8)	47 (69.1)	16 (64)	
Lymph node					
0	82	23 (62.2)	45 (57.7)	14 (48.3)	0.676
1−3	42	8 (21.6)	24 (30.8)	10 (34.5)	
≥4	20	6 (16.2)	9 (11.5)	5 (17.2)	
Stage					
0	20	7 (18.9)	9 (11.8)	4 (13.8)	0.475
I	38	11 (29.7)	19 (25)	8 (27.6)	
II	58	12 (32.5)	37 (48.7)	9 (31.0)	
III	26	7 (18.9)	11 (14.5)	8 (27.6)	
ER status					
Negative	45	15 (38.5)	23 (32.4)	7 (29.2)	0.715
Positive	89	24 (61.5)	48 (67.6)	17 (70.8)	
PR status					
Negative	53	17 (43.6)	28 (39.4)	8 (33.3)	0.721
Positive	81	22 (56.4)	43 (60.6)	16 (66.7)	
Her2/neu					
Negative	59	13 (33.3)	35 (49.3)	11 (45.8)	0.267
Positive	75	26 (66.7)	36 (50.7)	13 (54.2)	

ER = estrogen receptor; PR = progesterone receptor.

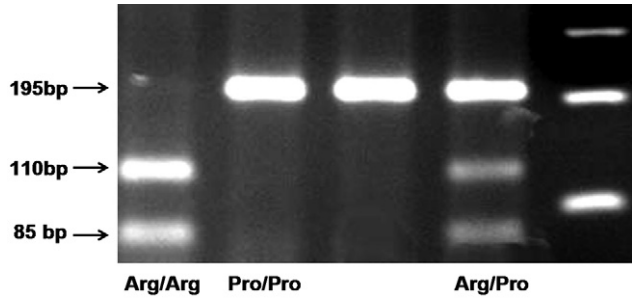

Figure 1. The p53-P/P72 variant was identified by a single band (195 bp), the p53-R/R72 variant produced two bands (85 bp and 110 bp), and the heterozygous p53-R/P72 variant displayed three bands (195, 85, and 115 bp).

Discussion

Among the 170 breast cancer cases we genotyped, there were 31 p53-P/P72 (31 of 170, 18.2%), 93 p53-R/P72 (93 of 170, 54.7%), and 46 p53-R/R72 (46 of 170, 27.1%), respectively. The allele frequencies were 0.54 for the p53-R72 and 0.46 for p53-P72 with the polymorphism in Hardy-Weinberg equilibrium. Among the analyzed clinicopathologic characteristics, we observed a statistically significant

association between the p53 codon 72 genotypes and tumor size but not with any other characteristics such as menopausal status, histologic type, tumor size, lymph node status, or histologic grade (Table 1). In addition, 55.2% of patients (16 of 29) with p53-R/R72 genotype had a t1 status compared with 30.9% or 36% of patients (21 of 68 and 9 of 25, respectively) in the P53-P/R72 or P53-R/R72 groups, respectively ($p = 0.046$). The association of p53 genotypes and breast cancer clinicopathologic features is inconsistent. Previous studies reported that the p53-P/P72 homozygotes seemed to present more often with lobular carcinoma and grade 1 tumors [26] , that they were more likely to have a positive axillary lymph node status [29], and that they were significantly associated with ER-positive breast cancer risk in postmenopausal women [31]. The reason that p53-P/R72 genotypes are associated with specific clinicopathologic features of breast cancer is currently unknown. Although some studies supported the finding that the P53-R72 allele represents a significant risk in tumorigenesis [13—17], it has been reported that patients with the p53-72P allele are more susceptible to cancer development and a poor clinical outcome [26]. Recently, Ozeki et al. [32] demonstrated a novel molecular difference underlying the tumor-suppressing function of the P53-P/R72 variants and this may at least partially explain why p53-R72 is associated with smaller tumors. They showed significantly diminished Mdm2-mediated degradation of p53-72R compared with p53-P72, which is associated with higher levels of phosphorylation at Ser-20 in p53-R72. They also showed enhanced p21 expression in p53-R72-expressing cells, which is dependent on phosphorylation at Ser-6 [32].

Although it has been proposed that there are inherent differences in the relative prevalence of the polymorphism in various populations [17,33], it is interesting to find a discrepancy in the distributions of the p53 codon 72 polymorphism between Asian and Caucasian populations (Table 2). There was more p53-P72 allele (40.6% in Asians vs. 26.4% in Caucasians) and there was twice the incidence of p53-P/P72 homozygotes (18% vs. 8%) among the Asian population. The homogeneity of the incidence of each genotype among different areas of Asia suggests consistency in this result. Compatible with the p53 R72P polymorphism locating at the proline-rich domain, which is responsible for the growth suppression and apoptosis activity, p53-P72 was less efficient in suppressing cell transformation, slower to induce apoptosis [34], and less efficient at binding and inactivating p73, which is a tumor suppressor protein responsible for apoptosis [14]. The reason for the greater prevalence of the p53-P72 allele in

Table 2 The distribution of p53 genotypes in Asian and Caucasian populations.

	p53-P/P72 (%)	p53-R/P72 (%)	p53-R/R72 (%)	Risk factors	Prognostic factors	Predictive factors
Caucasian						
Papadakis et al. [22]	12 (21)	10 (18)	34 (61)	N/A		
Langerød et al. [38]	26 (6.7)	136 (34.9)	228 (58.4)	N/A		
Bonafe et al. [25]	6 (8.9)	29 (43.3)	32 (47.8)	N/A	AR[a]	
Suspitsin et al. [24]	39 (9)	169 (38)	240 (54)	Against		
Tommiska et al. [26]	63 (7.3)	336 (39.2)	459 (53.5)	Against	**p53-P/P72**[b]	
Kalemi et al. [35]	3 (7)	13 (31)	26 (62)	p53-R/R72[c]		
Wegman et al. [39]	17 (7.7)	77 (35)	128 (57)	N/A		P53-P72[d]
Schmidt et al. [40]	618 (7.4)	3228 (32.7)	4499 (53.9)	Against		
Subtotal	784 (7.5)	3998 (38.3)	5646 (54.1)			
Asia						
Tsai et al.[30]	36 (18)	100 (50.0)	64 (32.0)	p53-P/P72[e]		
Noma et al. [31]	29 (15.2)	69 (36.1)	93 (48.7)	p53-P/P72[f]		p53-P/P72[f]
Xu [29]	23 (21)	55 (50)	32 (29)	N/A	**p53-P/P72**[g]	p53-P/P72[h]
Ma [37]	77 (19.1)	178 (44.1)	149 (36.8)	p53-P/P72[i]		
Chen	31 (18.2)	93 (54.7)	46 (27.1)			
Subtotal	194 (18)	485 (45.2)	396 (36.8)			

[a] Arginine allele is an independent prognostic factor for disease free survival (DFS) ($p = 0.039$) and overall survival (OS) ($p = 0.032$).
[b] p53—P/P72 homozygote was an independent poor prognostic factor (risk ratio of death, 2.1; 95% confidence interval,1.4—3.3; $p = 0.001$).
[c] Odds ratio for p53-R72 homozygotes to non- p53-R72 homozygote was 6.66, $p = 0.0001$ at 95% confidence interval 2.63—16.9.
[d] One p53-P72 allele had better distant recurrence-free survival when randomized to tamoxifen compared with those who were not ($p = 0.0033$).
[e] Significantly increased risk for p53-P72 homozygotes (odds ratio = 2.14; 95% confidence interval = 1.21—3.79).
[f] p53-P72 homozygotes were significantly associated with estrogen receptor-positive breast cancer risk in postmenopausal women (adjusted odds ratio = 3.42, $p < 0.01$).
[g] p53-P72 homozygotes have more positive axillary lymph node status ($p = 0.007$).
[h] p53-P72 homozygotes to be a strong predictor of poor pathologic response to neoadjuvant chemotherapy (odds ratio 6.7, 95% confidence interval,1.4—31.2; $p = 0.016$).
[i] *BP1* genotypes of T-885G and Gln1136Lys were associated with a significantly increased risk of breast cancer among p53-P72 homozygotes.

Asian patients with breast cancer remains unclear. In contrast to p53-R72 as a risk factor of breast cancer in a Caucasian [17,35] or Jewish population [36], p53-P/P72 homozygotes seem to be significantly associated with Asian breast cancer risk [23,31,37].

Conclusion

Our study suggested that the p53 codon 72 polymorphism in Taiwanese women is similar to that of the Asian population. We also found that p53-R/R72 phenotype was associated with smaller tumor. That the prevalence of the p53-P72 allele in Taiwan and Asia may elucidate the difference of the peak of the age-specific incidence rate of breast cancer in patients in Asian and Western countries warrants further research. Our preliminary results also need to be confirmed by a future study including a larger number of patients.

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